



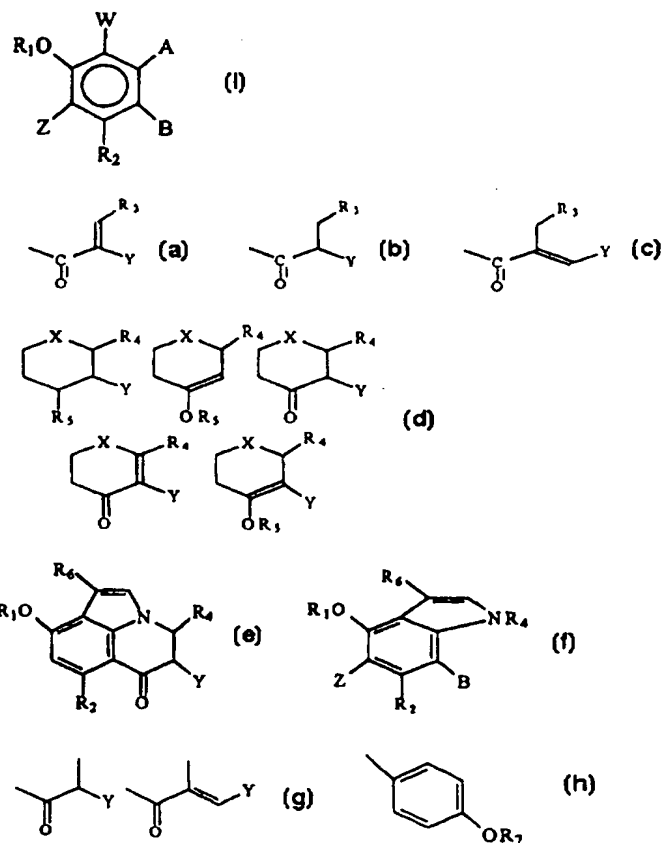
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(54) Title: THERAPEUTIC METHODS AND COMPOSITIONS INVOLVING ISOFLAVONES

(57) Abstract

Therapeutic methods of treatment, compositions and foodstuffs are described which contain isoflavone compounds described by general formula (I), in which Z is H, R₁ is H, or R_ACO where R_A is C₁₋₁₀alkyl or an amino acid, R₂ is H, OH, or OR_B where R_B is an amino acid, or COR_A where R_A is as previously defined, W is H, A is H or OH, and B is selected from (a), (b), (c), or W is H, and A and B taken together form a six-membered ring selected from (d), or W, A and B taken with the groups with which they are associated comprise (e), or W and A taken together with the groups with which they are associated comprise (f) and B is (g) wherein R₃ is H, COR_A where R_A is as previously defined, CO₂R_C where R_C is C₁₋₁₀alkyl, or COR_B where R_B is as previously defined, R₄ is H, COR_D where R_D is H, OH, C₁₋₁₀alkyl or an amino acid, CO₂R_C where R_C is as previously defined, COR_E where R_E is H, C₁₋₁₀alkyl or an amino acid, COOH, COR_C where R_C is as previously defined, or CONHR_E where R_E is as previously defined, R₅ is H, CO₂R_C where R_C is as previously defined, or COR_COR_E where R_C and R_E are as previously defined, and where the two R₅ groups are attached to the same group they are the same or different, R₆ is H or hydroxy C₁₋₁₀alkyl, X is preferably O, but may be N or S, and Y is (h) where R₇ is H, or C₁₋₁₀alkyl.



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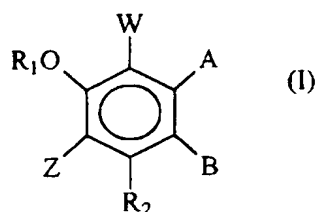
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THERAPEUTIC METHODS AND COMPOSITIONS INVOLVING ISOFLAVONES

This invention relates to therapeutic uses, methods, compounds, formulations, drinks and food
5 stuffs involving, containing, comprising and/or including certain isoflavone compounds.

The isoflavone compounds according to this invention are described by general formula I.



in which

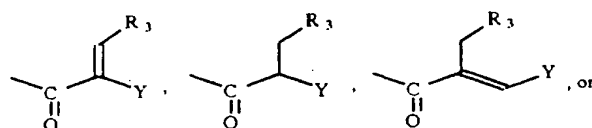
10 Z is H,

R₁ is H, or R_ACO where R_A is C₁₋₁₀ alkyl or an amino acid,

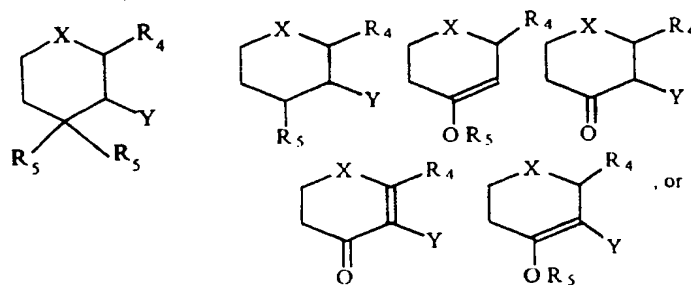
R₂ is H, OH, or OR_B where R_B is an amino acid or COR_A where R_A is as previously defined,

W is H, A is H or OH, and B is selected from

15

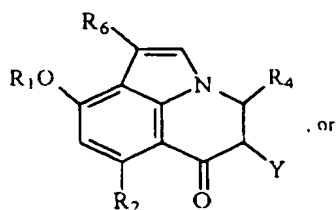


W is H, and A and B taken together form a six membered ring selected from

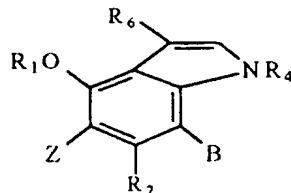


W, A and B taken with the groups with which they are associated comprise

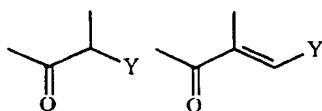
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W and A taken together with the groups with which they are associated comprise



5 and B is



wherein

R_3 is H, COR_A where R_A is as previously defined, CO_2R_C where R_C is C_{1-10} alkyl, or COR_B where R_B is as previously defined,

10 R_4 is H, COR_D where R_D is H, OH, C_{1-10} alkyl or an amino acid, CO_2R_C where R_C is as previously defined, COR_E where R_E is H, C_{1-10} alkyl or an amino acid, COOH, COR_C where R_C is as previously defined, or $CONHR_E$ where R_E is as previously defined, R_5 is H, CO_2R_C where R_C is as previously defined, or

COR_COR_E where R_C and R_E

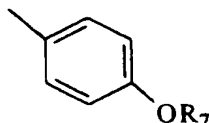
15 are as previously defined, and where the two R_s groups are attached to the same group they are the same or different,

R_6 is H or hydroxy C_{1-10} alkyl,

X is preferably O, but may be N or S, and

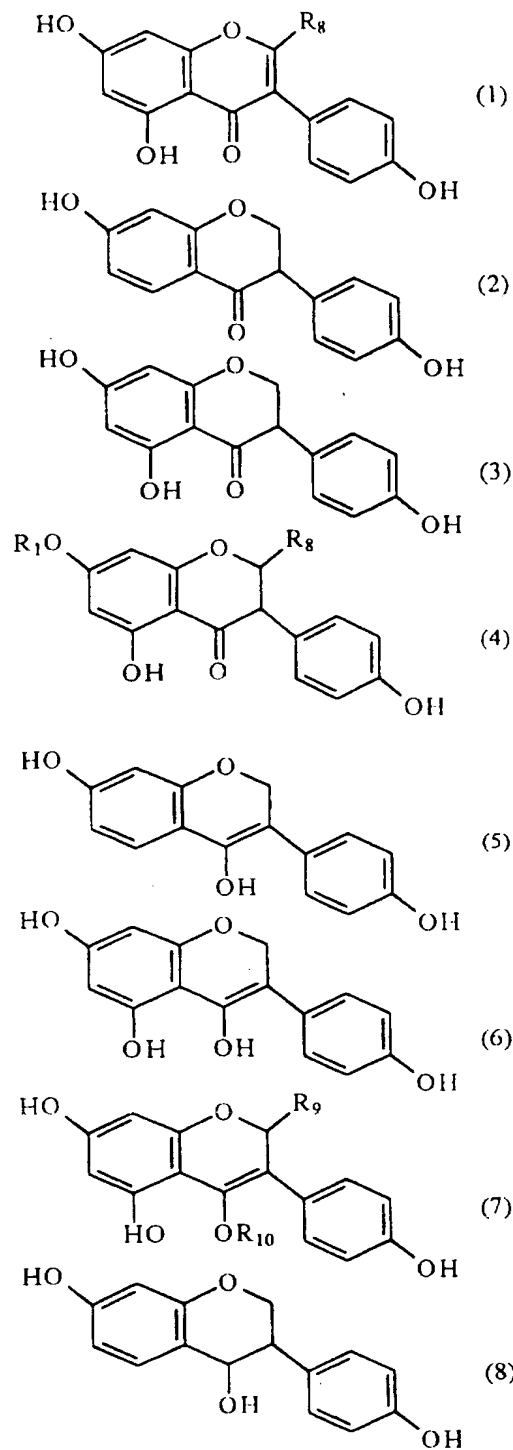
Y is

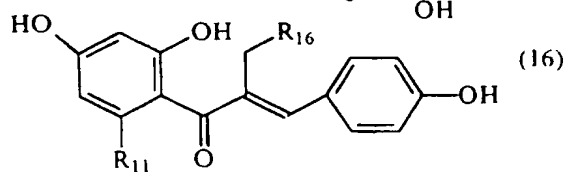
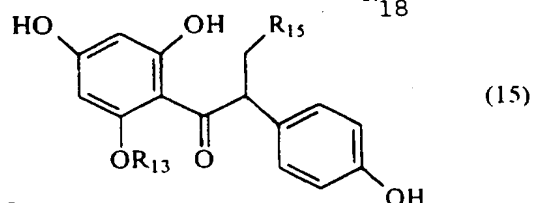
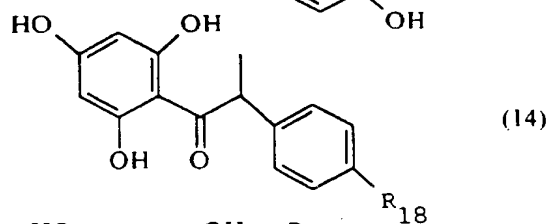
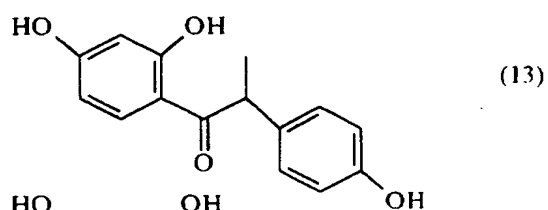
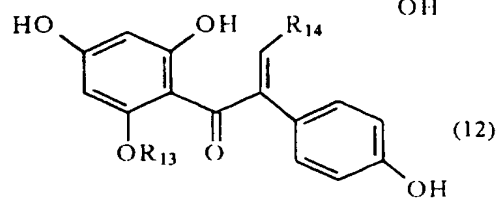
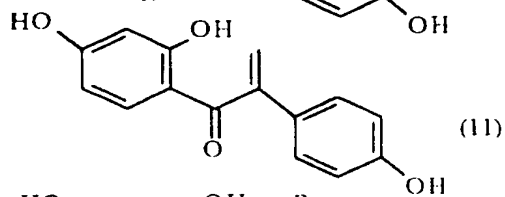
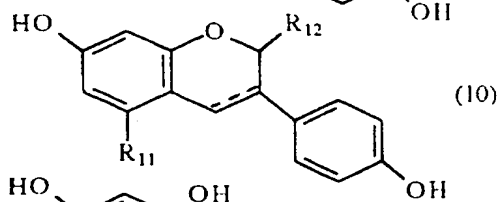
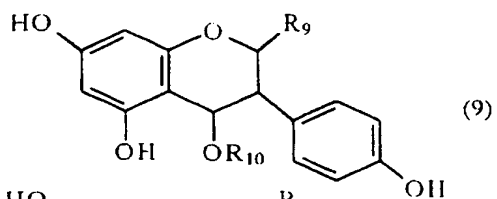
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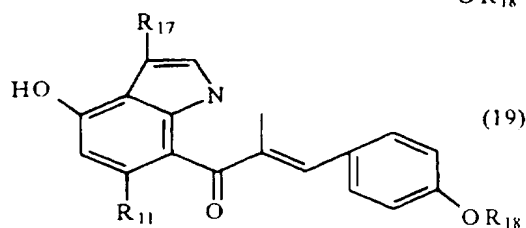
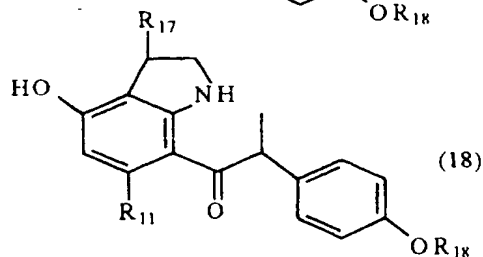
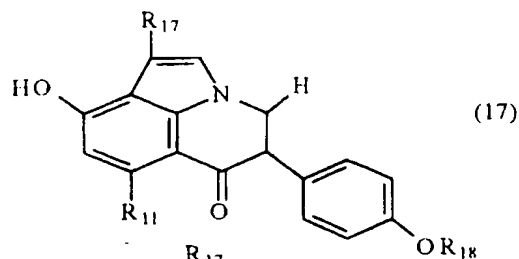


where R_7 is H, or C_{1-10} alkyl.

Preferably the compounds of the formula I are selected from:







wherein

R_8 is COR where R_D is as previously defined,

R_9 , CO_2R_C or COR_E where R_C and R_E are as previously defined,

5 R_{10} is COR_C or COR_COR_E where R_C and R_E are as previously defined,

R_{11} is H or OH,

R_{12} is H, COOH, CO_2R_C where R_C is as previously defined, or $CONHR_E$ where R_E is as previously defined,

R_{13} is OH, OR_B where R_B is as previously defined, or COR_A where R_A is as previously defined,

10 R_{14} is H, or COR_A where R_A is as previously defined,

R_{15} is COR_A where R_A is as previously defined,

R_{16} is H, COR_B or CO_2R_C where R_B and R_C are as previously defined,

R_{17} is H or hydroxy C_{1-10} alkyl,

R_{18} is H or C_{1-10} alkyl,

15 and "—" represents either a single bond or a double bond.

Alkyl groups may be straight or branched chains. C_{1-10} alkyl preferably contains from 1 to 5 carbons, more preferably methyl, ethyl or propyl.

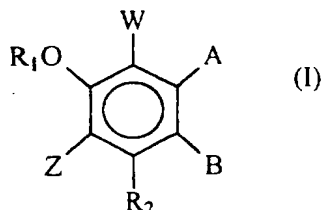
Certain of the above compounds may be referred to by the names dihydrodaidzein (compound 1 where R_8 is H), dihydrogenestein (compounds 2 and 5), dehydro-O-desmethylangolensin (compound 11), tetrahydrodaidzein (compound 8), equol and dehydroequol (compound 10), O-desmethyl-angolensin (ODMA - compound 13), and 6-hydroxy-O-desmethylangolensin (6-hydroxy-ODMA - compound 14).

It has surprisingly been found by the inventors that compounds of the formula I, and more specifically compounds of the formulae 1 to 19 have particular utility and effectiveness in the treatment, prophylaxis, amelioration defence against, and/or prevention of menopausal syndrome including hot flushes, anxiety, and depression, mood swings, night sweats, headaches, and urinary incontinence; osteoporosis; premenstrual syndrome, including fluid retention, cyclical mastalgia, and dysmenorrhoea; Reynaud's Syndrome; Reynaud's Phenomenon; Buerger's Disease; coronary artery spasm; migraine headaches; hypertension; benign prostatic hypertrophy; breast cancer; uterine cancer; ovarian cancer; testicular cancer; large bowel cancer; endometrial cancer; prostatic cancer; uterine cancer; atherosclerosis; Alzheimers disease; inflammatory diseases including inflammatory bowel disease, ulcerative colitis, Crohns disease; rheumatic diseases including rheumatoid arthritis; acne; baldness including male pattern baldness (alopecia hereditaria); psoriasis and diseases associated with oxidant stress including cancer, myocardial infarction stroke, arthritis, sunlight induced skin damage or cataracts.

According to a first aspect of the present invention there is provided a method for the treatment, prophylaxis, amelioration, defence against, and/or prevention of menopausal syndrome including hot flushes, anxiety, and depression, mood swings, night sweats, headaches, and urinary incontinence; osteoporosis; premenstrual syndrome, including fluid retention, cyclical mastalgia, and dysmenorrhoea; Reynaud's Syndrome; Reynaud's Phenomenon; Buerger's Disease; coronary artery spasm; migraine headaches; hypertension; benign prostatic hypertrophy; breast cancer; uterine cancer; ovarian cancer; testicular cancer; large bowel cancer; endometrial cancer; prostatic cancer; uterine cancer; atherosclerosis; Alzheimers disease; inflammatory diseases including inflammatory bowel disease, ulcerative colitis, Crohns disease; rheumatic diseases including rheumatoid arthritis; acne; baldness

including male pattern baldness (alopecia hereditaria); psoriasis and diseases associated with oxidant stress including cancer, myocardial infarction stroke, arthritis, sunlight induced skin damage or cataracts which comprises administering to a subject a therapeutically effective amount of one or more compounds of the formula I:

5



where R₁, R₂, Z, W, A and B are as previously defined, either alone or in association with one or more pharmaceutically acceptable carriers and/or excipients.

- 10 Preferably, one or more compounds of the formulae 1 to 19 may be used in the treatment, prophylaxis, amelioration of menopausal syndrome including hot flushes, anxiety, and depression, mood swings, night sweats, headaches, and urinary incontinence; osteoporosis; premenstrual syndrome, including fluid retention, cyclical mastalgia, and dysmenorrhoea; Reynaud's Syndrome; Reynaud's Phenomenon; Buerger's Disease; coronary artery spasm;
- 15 migraine headaches; hypertension; benign prostatic hypertrophy; breast cancer; uterine cancer; ovarian cancer; testicular cancer; large bowel cancer; endometrial cancer; prostatic cancer; uterine cancer; atherosclerosis; Alzheimers disease; inflammatory diseases including inflammatory bowel disease, ulcerative colitis, Crohns disease; rheumatic diseases including rheumatoid arthritis; acne; baldness including male pattern baldness (alopecia hereditaria);
- 20 psoriasis and diseases associated with oxidant stress including cancer, myocardial infarction stroke, arthritis, sunlight induced skin damage or cataracts (for convenience hereafter referred to as the "therapeutic indications"). Cancer, myocardial infarction, stroke, arthritis, sunlight induced skin damage and cataracts are generally regarded to be associated with oxidant stress. This invention includes the treatment of diseases associated with oxidant stress.

25

A second aspect of the present invention is the use of compounds of the formula I for the manufacture of a medicament for the treatment, amelioration, defence against, prophylaxis and/or prevention of one or more of the therapeutic indications. It is particularly preferred that one or more compounds of the formulae 1 to 19 are employed in the treatment, prophylaxis, amelioration, defence against, and/or prevention of said indications.

A third aspect of the present invention is use of one or more compounds of the formula I in the treatment, amelioration, defence against, prophylaxis and/or prevention of one or more of the therapeutic indications. Compounds of the formulae 1 to 19 are particularly preferred.

A fourth aspect of the present invention comprises an agent for the treatment, prophylaxis, amelioration, defence against and/or treatment of the therapeutic indications which comprises one or more compounds of the formula I either alone or in association with one or more carriers or excipients. Compounds of the formulae 1 to 19 are particularly preferred.

A fifth aspect of the invention is a therapeutic composition which comprises one or more compounds of the formula I in association with one or more pharmaceutical carriers and/or excipients. It is preferred that the compositions comprise one or more compounds of the formulae 1 to 19.

A sixth aspect of the present invention is a drink or food-stuff, which contains one or more compounds of the formula I. Preferably the food stuff contains one or more compounds of the formulae 1 to 19.

A seventh aspect of the present invention is a microbial culture or a food-stuff containing one or more microbial strains which microorganisms produce one or more compounds of the formula I. Preferably said microorganisms produce one or more of the compounds of the formulae 1 to 19.

An eighth aspect of the present invention relates to one or more microorganisms which produce one or more compounds of the formula I. Preferably the microorganism is a purified culture,

which may be admixed and/or administered with one or more other cultures which produce compounds of the formula I. The compounds of the formula I preferably are selected from one or more of compounds of the formulae 1 to 19.

- 5 In a further aspect this invention is directed to compounds of the formula I. Preferably said compounds comprise compounds of the formulae 1 to 19.

Compounds of the present invention have particular application in the treatment of diseases associated with or resulting from estrogenic effects androgenic effects, vasolidatory and
10 spasmodic effects, inflammatory effects and oxidative effects.

The amount of the compound of the formula I which is required in a therapeutic treatment according to the invention will depend upon a number of factors, which include the specific application, the nature of the particular compound used, the condition being treated, the mode
15 of administration and the condition of the patient. In general, a daily dose per patient is in the range of 0.1 mg to 2 g; typically from 0.5 mg to 1 g; preferably from 50 mg to 200 mg.

Compounds of the formula I may be in a manner and amount as is conventionally practised. See, for example, Goodman and Gilman, *The Pharmacological Basis of Therapeutics*, 1299
20 (7th Edition, 1985). The specific dosage utilised will depend upon the condition being treated, the state of the subject, the route of administration and other well known factors as indicated above.

The production of a pharmaceutical composition for the treatment of the therapeutic indications
25 herein described (for convenience hereafter referred to as the "active compounds") are typically admixed with one or more pharmaceutically or veterinarily acceptable carriers and/or excipients as are well known in the art.

The carrier must, of course, be acceptable in the sense of being compatible with any other
30 ingredients in the formulation and must not be deleterious to the subject. The carrier or excipient may be a solid or a liquid, or both, and is preferably formulated with the compound

as a unit-dose, for example, a tablet, which may contain from 0.5% to 59% by weight of the active compound, or up to 100% by weight of the active compound. One or more active compounds may be incorporated in the formulations of the invention, which may be prepared by any of the well known techniques of pharmacy consisting essentially of admixing the components, optionally including one or more accessory ingredients.

The formulations of the invention include those suitable for oral, rectal, optical, buccal (for example, sublingual), parenteral (for example, subcutaneous, intramuscular, intradermal, or intravenous) and transdermal administration, although the most suitable route in any given case will depend on the nature and severity of the condition being treated and on the nature of the particular active compound which is being used.

Formulation suitable for oral administration may be presented in discrete units, such as capsules, cachets, lozenges, or tablets, each containing a predetermined amount of the active compound; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion. Such formulations may be prepared by any suitable method of pharmacy which includes the step of bringing into association the active compound and a suitable carrier (which may contain one or more accessory ingredients as noted above). In general, the formulations of the invention are prepared by uniformly and intimately admixing the active compound with a liquid or finely divided solid carrier, or both, and then, if necessary, shaping the resulting mixture such as to form a unit dosage. For example, a tablet may be prepared by compressing or moulding a powder or granules containing the active compound, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing, in a suitable machine, the compound of the free-flowing, such as a powder or granules optionally mixed with a binder, lubricant, inert diluent, and/or surface active/dispersing agent(s). Moulded tablets may be made by moulding, in a suitable machine, the powdered compound moistened with an inert liquid binder.

Formulations suitable for buccal (sublingual) administration include lozenges comprising the active compound in a flavoured base, usually sucrose and acacia or tragacanth; and pastilles comprising the compound in an inert base such as gelatin and glycerin or sucrose and acacia.

5 Compositions of the present invention suitable for parenteral administration conveniently comprise sterile aqueous preparations of the active compounds, which preparations are preferably isotonic with the blood of the intended recipient. These preparations are preferably administered intravenously, although administration may also be effected by means of subcutaneous, intramuscular, or intradermal injection. Such preparations may conveniently be
10 prepared by admixing the compound with water or a glycine buffer and rendering the resulting solution sterile and isotonic with the blood. Injectable formulations according to the invention generally contain from 0.1% to 60% w/v of active compound and are administered at a rate of 0.1 ml/minute/kg.

15 Formulations suitable for rectal administration are preferably presented as unit dose suppositories. These may be prepared by admixing the active compound with one or more conventional solid carriers, for example, cocoa butter, and then shaping the resulting mixture.

Formulations or compositions suitable for topical administration to the skin preferably take the
20 form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers which may be used include Vaseline, lanoline, polyethylene glycols, alcohols, and combination of two or more thereof. The active compound is generally present at a concentration of from 0.1% to 0.5% w/w, for example, from 0.5% to 2% w/w. Examples of such compositions include cosmetic skin creams.

25 Formulations suitable for transdermal administration may be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Such patches suitably contain the active compound as an optionally buffered aqueous solution of, for example, 0.1 M to 0.2 M concentration with respect to the said active
30 compound.

Formulations suitable for transdermal administration may also be delivered by iontophoresis (see, for example, *Pharmaceutical Research* 3 (6), 318 (1986)) and typically take the form of an optionally buffered aqueous solution of the active compound. Suitable formulations comprise citrate or bis/tris buffer (pH 6) or ethanol/water and contain from 0.1 M to 0.2 M active ingredient.

The active compounds may be provided in the form of food stuffs, such as being added to, admixed into, coated, combined or otherwise added to a food stuff. The term food stuff is used in its widest possible sense and includes liquid formulations such as drinks including dairy products and other foods, such as health bars, desserts, etc. Food formulations containing compounds of the invention can be readily prepared according to standard practices.

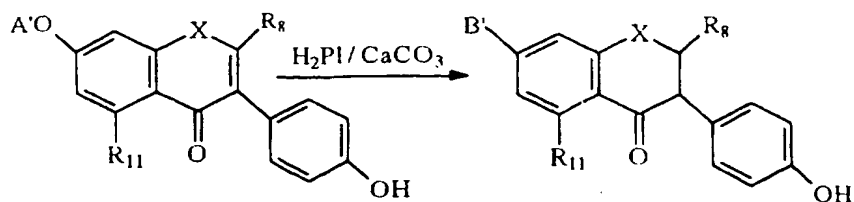
Compounds of the present invention have potent antioxidant activity and thus find wide application in pharmaceutical and veterinary uses, in cosmetics such as skin creams to prevent skin ageing, in sun screens, in foods, health drinks, shampoos, and the like.

It has surprisingly been found that compounds of the formula I interact synergistically with vitamin E to protect lipids, proteins and other biological molecules from oxidation. Accordingly, a further aspect of this invention provides a composition comprising one or more compounds of the formula I, vitamin E, and optionally a pharmaceutically, veterinarily or cosmetically acceptable carriers and/or excipients.

Therapeutic methods, uses and compositions may be for administration to humans or animals, such as companion and domestic animals (such as dogs and cats), birds (such as chickens, turkeys, ducks), livestock animals (such as cattle, sheep, pigs and goats) and the like.

Compounds of the formula I may be prepared as follows:

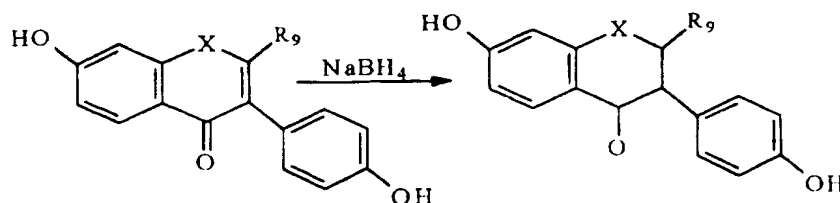
- A. Hydrogenation of daidzein, genistein or derivatives thereof using palladium on calcium carbonate, as follows



where A' is H or R₁ where R₁ is as previously defined and R₈ and R₁₁ and X are as previously defined. Compounds 2, 3, 4, 5, 6 and 7 may be produced by this method. Compounds 5 to 7 are enol forms of compounds 2 to 4.

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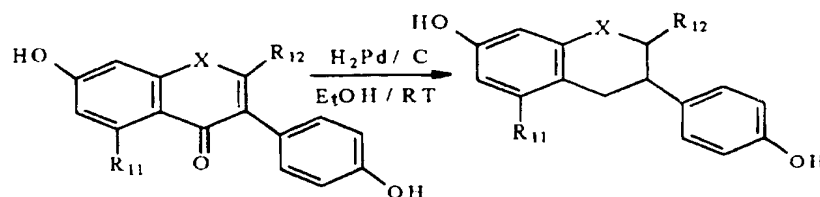
B. Reduction of daidzein and daidzein derivatives with sodium borohydride as follows:



where R₉ and X are as previously defined. Compound 8 may be produced by this method.

10

C. Hydrogenation of daidzein and diadzein derivatives using palladium on charcoal as a catalyst.

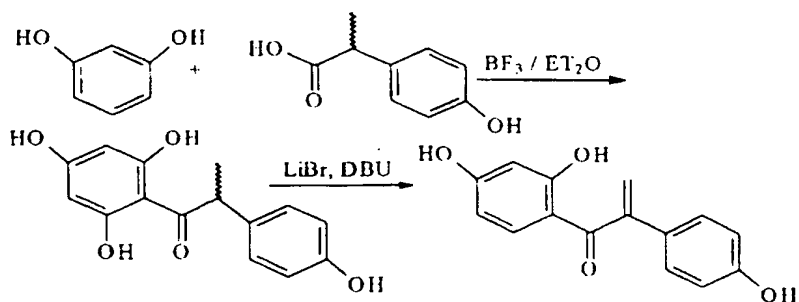


where R₁₁ and R₁₂ are as previously defined. Compound 10 may be produced by this method.

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D. Acylation of resorcinol or derivatives thereof, followed by dehydrogenation with lithium bromide

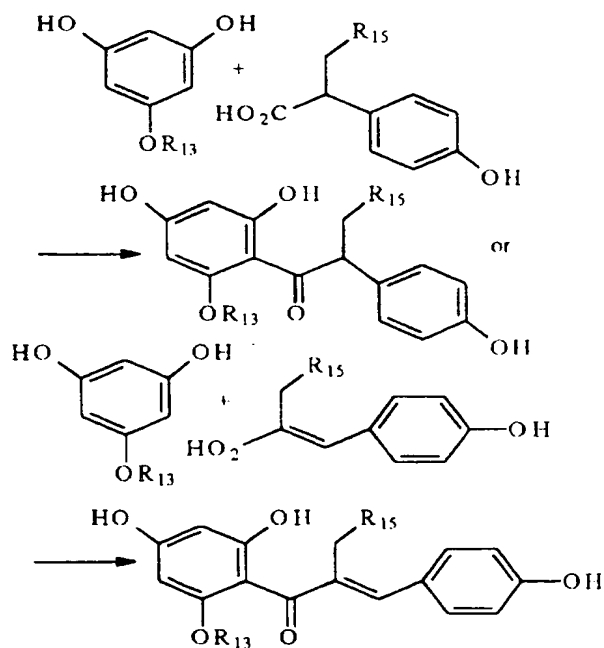
14.



Compounds 11 and 14 may be produced by this method. Compound 12 may be produced in a similar manner.

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- E. Acylation of 1, 3 and 5 trisubstituted benze with 4-hydroxyphenyl isopropyl acid or derivatives thereof.

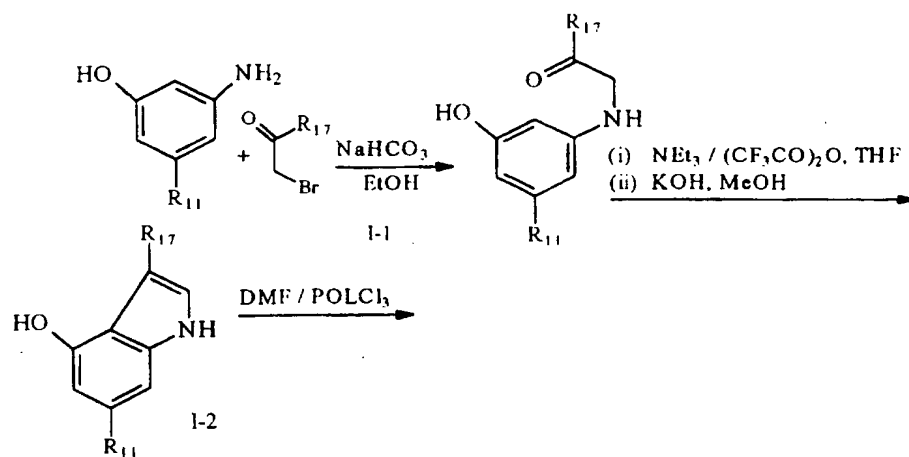


where R_{13} and R_{15} are as previously defined. Compounds 15 and 16 may be produced by this method.

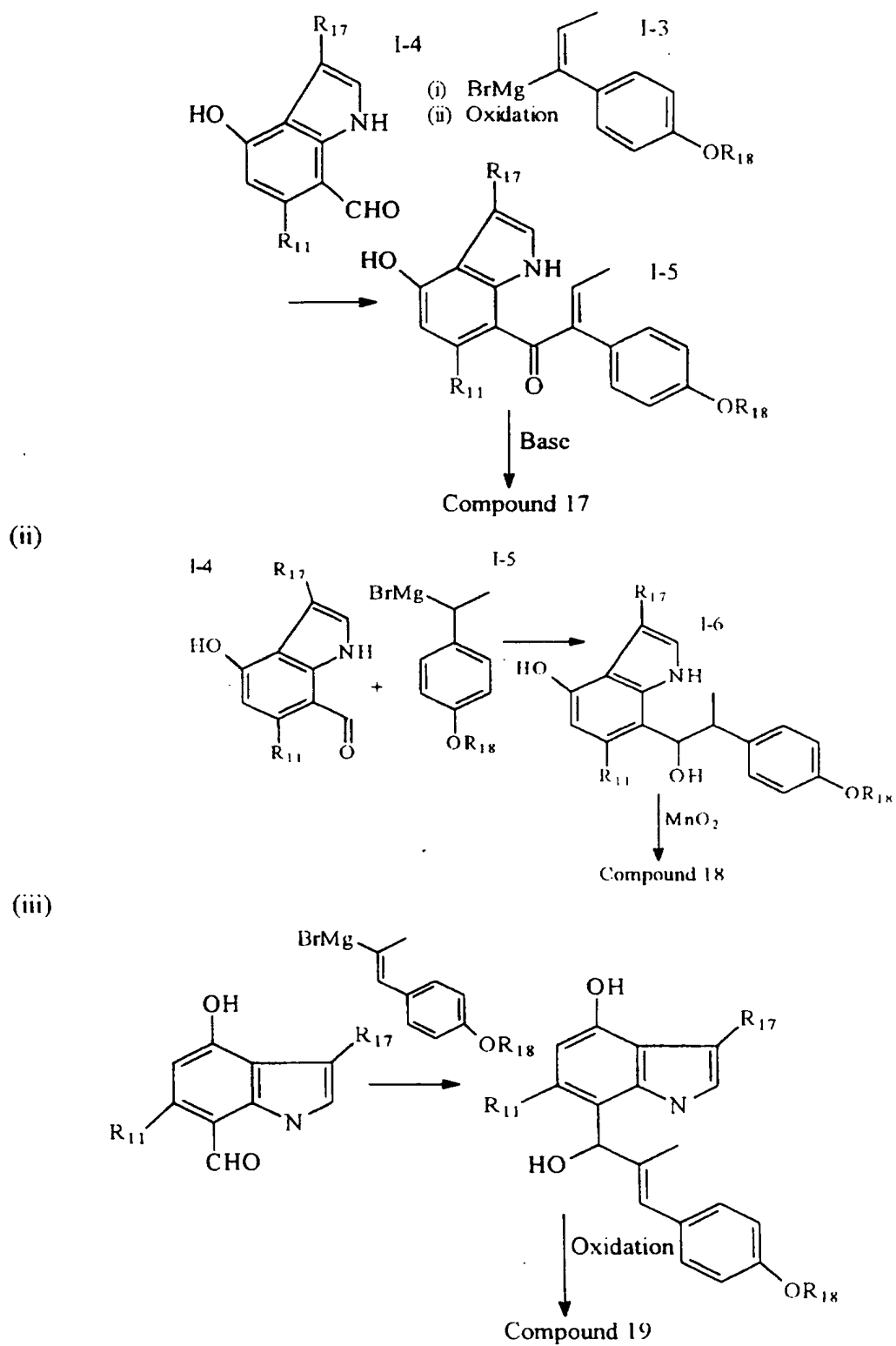
10

- F. Compounds of the formulae 17, 18 and 19 may be prepared according to the following reaction schemes.

(i)



16



where R₁₁, R₁₇ and R₁₈ are as previously defined.

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G. HPLC fractionation of human urine/urine fractions of HPLC/GLC fractionation of bacterial culture supernatant so as to give purified compounds of the formulae 1 to 19. Product identity is confirmed by mas spectrometry. Compounds of the formulae 1 to 19 may be purified according to Joannou *et al* (1995) *J. Steroid. Biochem. Molec. Biol.* 54, 167-184, which is incorporated herein by reference.

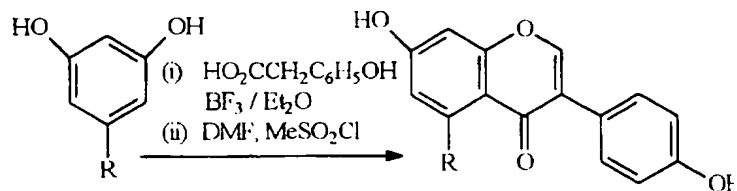
It has surprisingly been observed by the inventors that the presence of isoflavonoids, in bodily secretions, more particularly, isoflavonoid metabolites in the urine of subjects, is associated with a specific therapeutic response, medical condition, or absence of a specific medical condition. Determining the specific biological fingerprint of different isoflavonoids excreted by individuals enables therapeutic methods of treatment to be carried out.

Embodiments to the invention will now be described with reference to the following non-limiting Examples.

EXAMPLE 1

Daidzein and Genistein

Daidzein can be obtained by Friedel-Crafts acylation of resorcinol with 4-hydroxy-phenylacetic acid using boron trifluoride etherate as catalyst, then treated with DMF and methanesulfonyl chloride in 72% yield according to the method of Wähälä's (*Finnish Chem. Lett.* 1989, 16, 79). Although the genistein is commercially available, it is very expensive. However, it can be synthesized by the same method as daidzein, using 1,3,5-trihydroxybenzene instead of resorcinol.

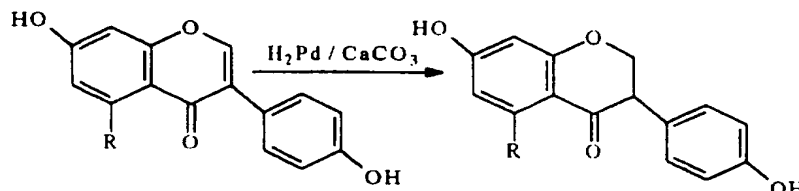


Where R is H the product is daidzein, when OH, genistein.

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Dihydrodaidzein and Dihydrogenistein (Compounds 2 and 3 respectively)

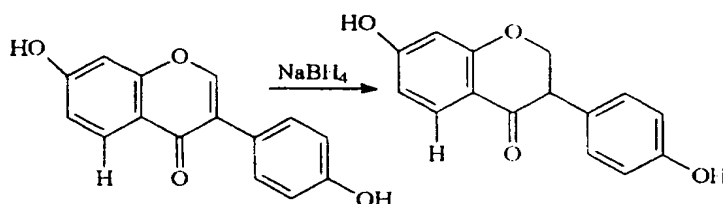
Hydrogenation of daidzein and genistein using palladium on calcium carbonate as a catalyst gives dihydrodaidzein and dihydrogenistein in good yield.



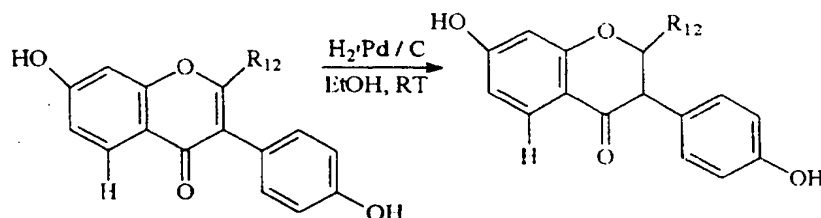
5 Where R is H the product is dihydrodaidzein, when OH, dihydrogenistein.

Tetrahydrodaidzein (Compound 8)

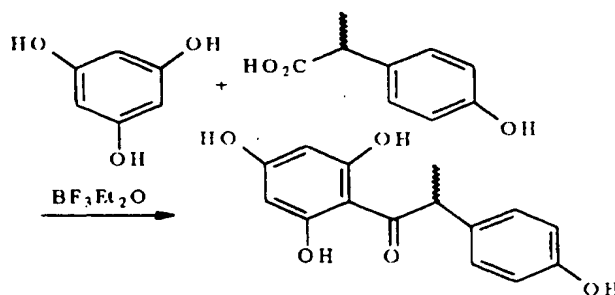
Reduction of daidzein with sodium borohydride gives the title compound.

10 Equol derivatives (Compound 10)

Equol derivatives are obtained from hydrogenation of daidzein derivatives using palladium on charcoal as a catalyst (*Finnish Chem Lett.* 1989, 16, 79).

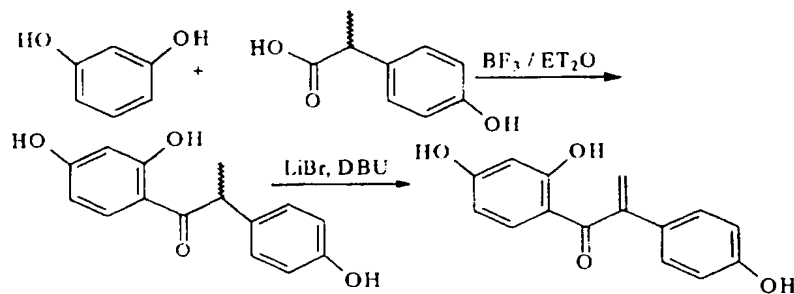
6-hydroxy-O-demethylangolensin (Compound 14)

15 4-hydroxyphenylisopropyl acid is acylated with 1,3,5-trihydroxybenzene to give the title compound.

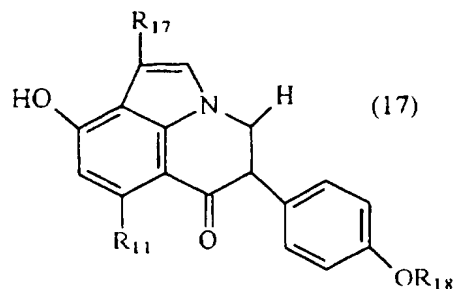


2-dehydro-O-demethylangolensin (Compound 11)

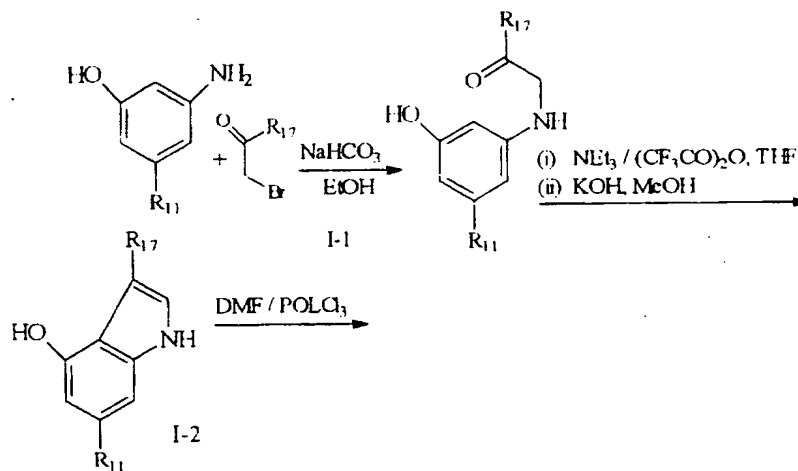
The title compound is obtained by acylation of resorcinol then dehydrogenation as shown below.

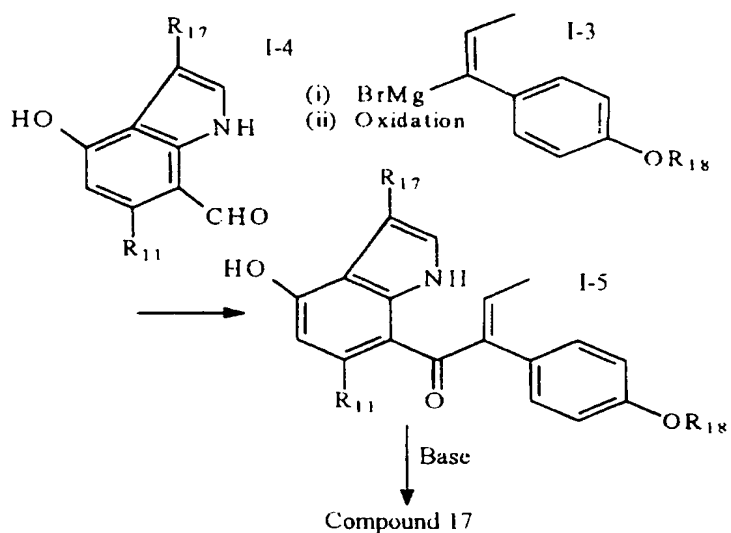


5 Compounds of the formulae 17, 18 and 19 are prepared as follows:

Compound 17

Compounds of the formula 17 are prepared according to the following reaction scheme



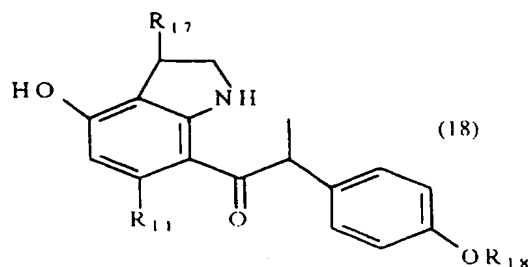


where R₁₁, R₁₇ and R₁₈ are as previously defined.

- 5 Indole I-2 is prepared as shown above according to the method of Black *et al* (*Aust. J. Chem.* 33 (1980) pages 343-350) which is incorporated herein by reference.

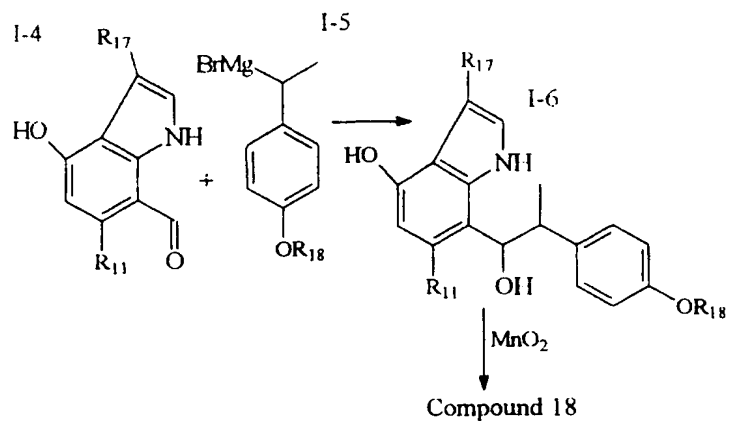
Indole I-4 is obtained by the Vilsmeier reaction. Electrophilic attack at 7-C position is preferred over that at 2-C position when there is an electron withdrawing group at the 3-C position. Nucleophilic addition of aldehyde with a Grignard reagent I-3 gives the secondary alcohol which on oxidation with MnO₂ will give the ketone I-5 and in mild base compounds of the formula 17.

Compound 18



Compounds of the formula 18 are prepared according to the following reaction scheme:

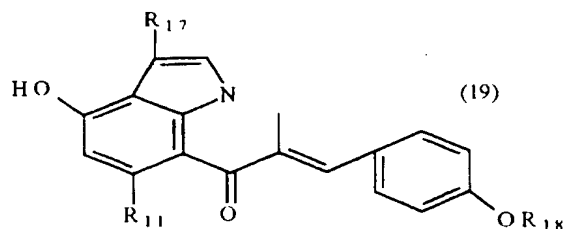
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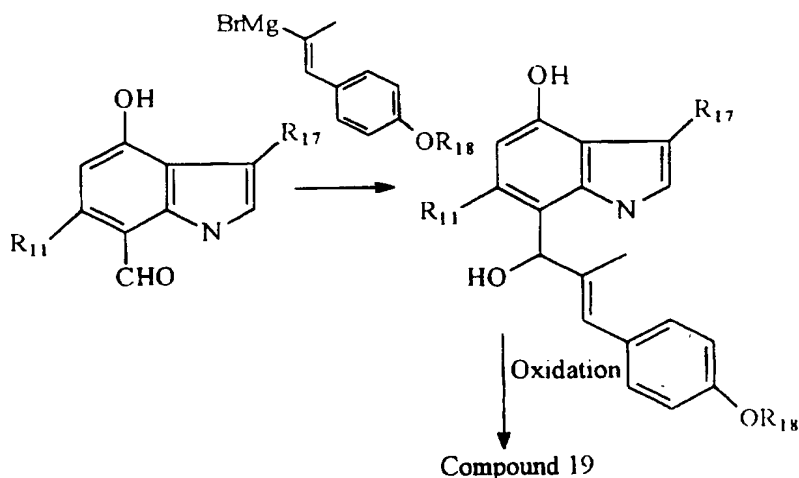
where R_{24} , R_{25} and R_{26} are as previously defined.

- 5 Nucleophilic addition of 7-aldehyde indole I-4 with Grignard reagent I-5 gives alcohol I-6 which on oxidation with MnO_2 gives compounds of the formula 18.

Compound 19



- 10 Compounds of the formula 19 are prepared according to the following reaction scheme.

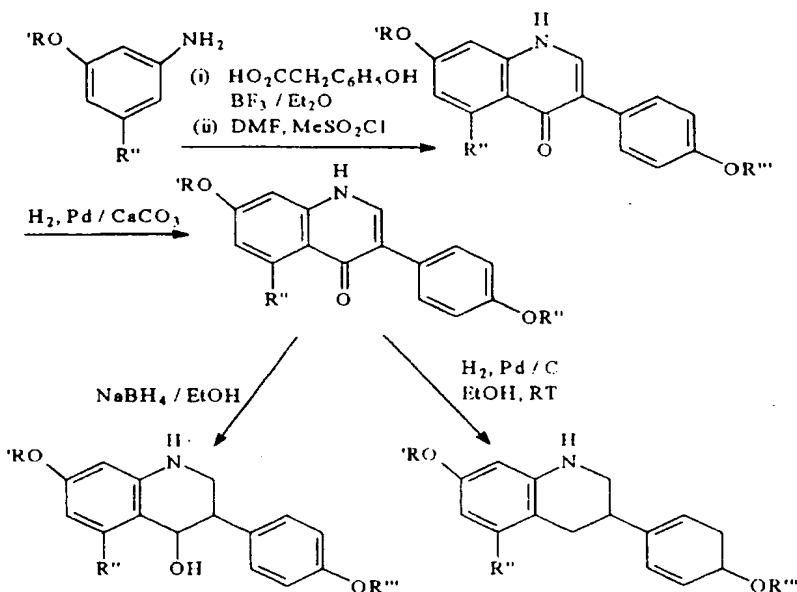


where R_{11} , R_{17} and R_{18} are as previously defined.

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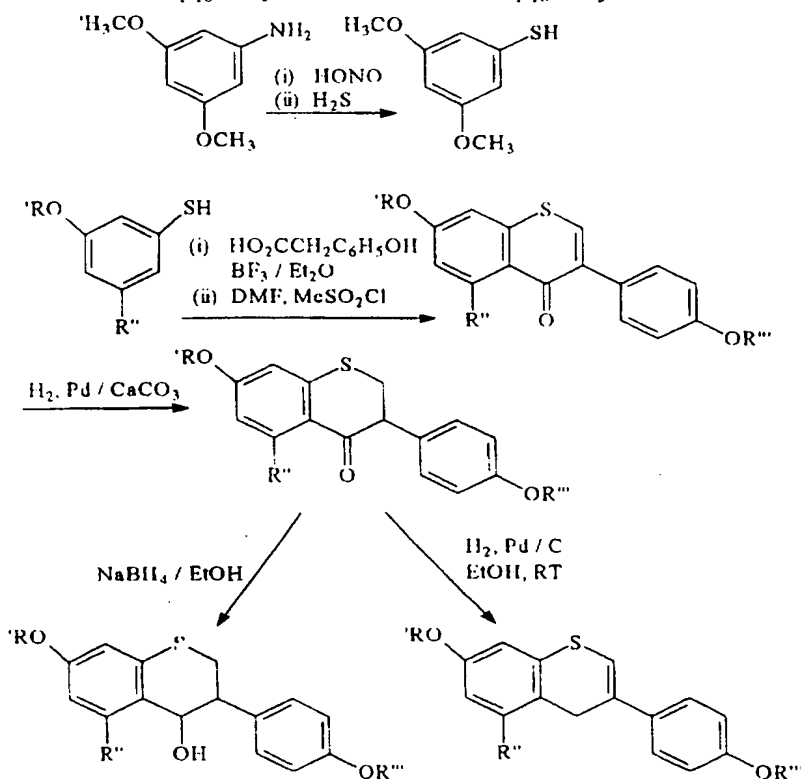
EXAMPLE 2

Nitrogen and sulphur containin heterocyclic ring systems are synthesized according to the following reaction schemes:



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where R' is H or $-OC_{1-10}$ alkyl, R'' is OH or OC_{1-10} alkyl, and R''' is H or OC_{1-10} alkyl.



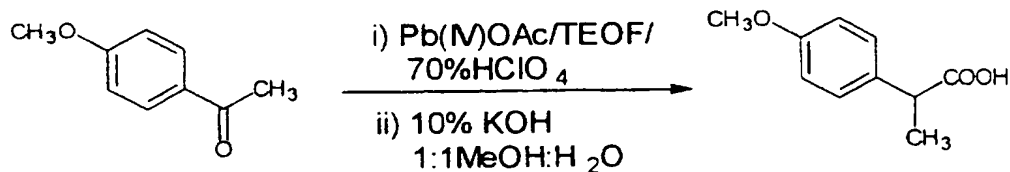
where R', R'' and R''' is as previously defined.

EXAMPLE 3

1: Synthesis of ODMA (O-Desmethylangolensin 2,4,4'-trihydroxyphenyl- α -methyl-desoxybenzoin). Compound 13.

1.1: 2-(*p*-Methoxyphenyl)propionic acid

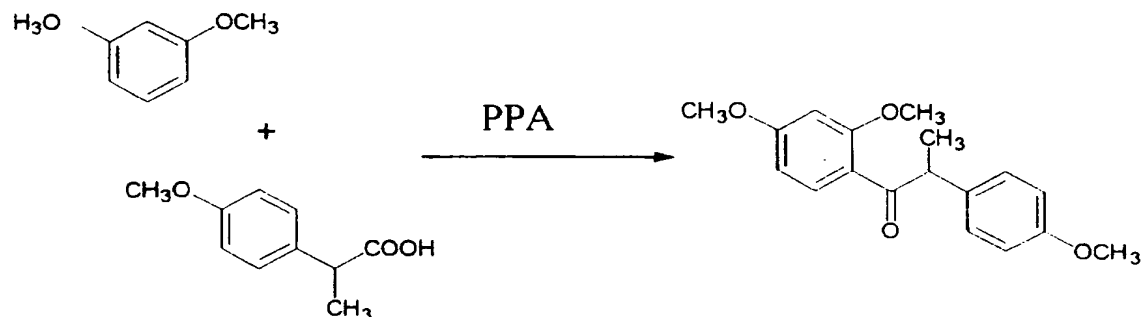
A mixture of *p*-methoxypropiophenone (2.39g, 14.5mmol), 90% lead(IV)acetate (6.45g, 14.5mmol), triethylorthoformate (15ml) and 70% perchloric acid (1.2ml, 29mmol) was heated to 55°C for 18h. The mixture was cooled and the triethylorthoformate removed under reduced pressure. The residue was dissolved in CHCl₃ and the remaining precipitate filtered off and discarded. The CHCl₃ solution was then washed with water and evaporated to yield the crude ester. This crude ester product was dissolved in a 10% KOH 1:1 water:methanol solution, which was then refluxed for 3h. After cooling the methanol was evaporated under reduced pressure and the aqueous solution washed with diethylether (3x25ml). The aqueous solution was acidified with 2N H₂SO₄, then washed again with diethylether (3x25ml). The combined fractions from the second ether wash were dried (Na₂SO₄) and evaporated to give the propionic acid (1.66g, 63%).



1.2: 2,4,4'-Trimethoxy- α -methyl-desoxybenzoin

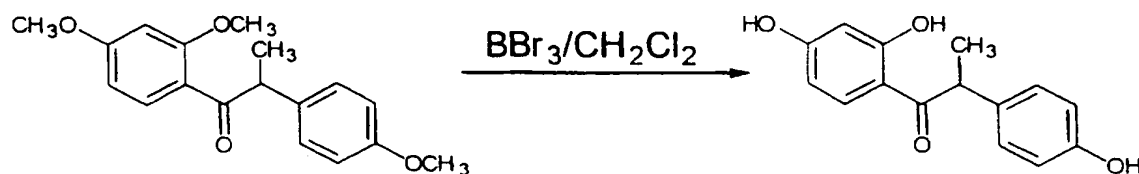
2-(*p*-Methoxyphenyl)propionic acid (0.39g, 4mmol) and 1,3-dimethoxybenzene (0.5g, 0.5ml, 4mmol) were mixed in polyphosphoric acid (PPA) (10g) and the reaction mixture was mechanically stirred at 75°C for 5h. The reaction mixture was then allowed to cool to room

temperature and mechanically stirred for a further 12h. The reaction was then quenched with ice water and the product extracted with CHCl_3 (3x25ml). The CHCl_3 layer was dried (Na_2SO_4) and the solvent removed under reduced pressure. The residual crude product was purified by silica gel column chromatography (eluent 7:2 CH_2Cl_2 :EtOAc) to give the pure 2,4,4'-trimethoxy- α -methyldesoxybenzoin (0.68g, 58%)



1.3 2,4,4'-Trihydroxyphenyl- α -methyldesoxybenzoin (O-Desmethylangolensin or O-DMA)

2,4,4'-Trimethoxy- α -methyldesoxybenzoin (0.312g 1.04mmol) was dissolved in dry CH_2Cl_2 (10ml). To this solution 5 equivalents of 1.0M BBr_3 in hexane (1.3g, 5.2ml, 5.2mmol) was added slowly and the reaction mixture allowed to stir under N_2 at room temperature for 6 days. Reaction was quenched with ice/water and after stirring for 1h the product was extracted with diethylether (3x25ml). The ether layer was dried (Na_2SO_4) and the solvent removed under reduced pressure. The residual crude product was purified by silica gel column chromatography (eluent 7:1 CH_2Cl_2 :EtOAc) to give the pure 2,4,4'-trihydroxyphenyl- α -methyldesoxybenzoin (0.68g, 58%)



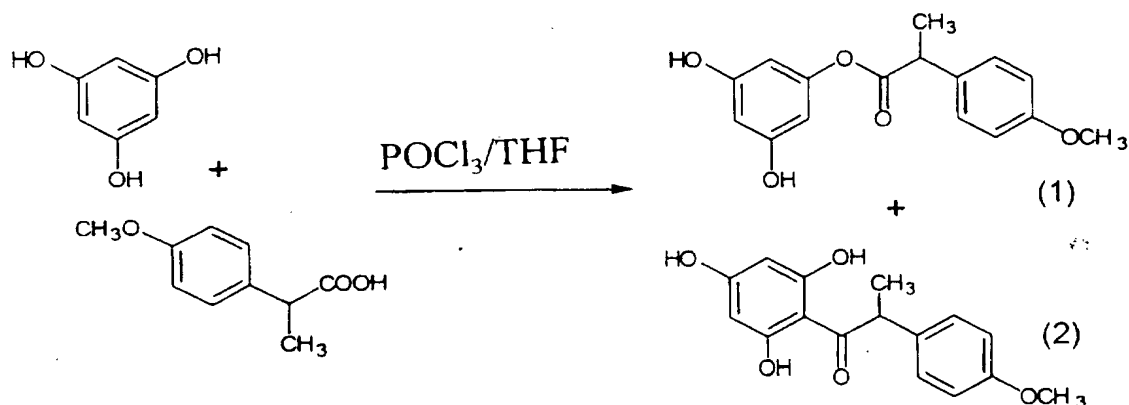
2: Synthesis of 4'methoxy 6-OH-ODMA (4'methoxy6-OH-O-Desmethylangolensin 2,4,6,4'-tetrahydroxyphenyl- α -methyldeoxybenzoin)

2.1: Use of POCl₃ with Phloroglucinol and the *p*-methoxy phenyl propionic acid

5

2-(*p*-Methoxyphenyl)propionic acid (0.1g, 0.55mmol) and 1.1 equivalents of 1,3,5-trihydroxybenzene (Phloroglucinol) (0.077g, 0.61mmol) were dissolved in dry tetrahydrofuran (THF) (2ml). Freshly distilled POCl₃ (1.0ml) was added to the solution and the reaction mixture was allowed to stir at room temperature for 4 days. The reaction was then quenched with ice water and the product extracted with diethylether (3x10ml). The ether layer was dried (Na₂SO₄) and the solvent removed under reduced pressure. The residual crude product was purified by silica gel column chromatography (eluent 7:2 CH₂Cl₂:EtOAc) to give two products namely, the ester (1) and the desired 4'methoxy-6-OH-ODMA (2).

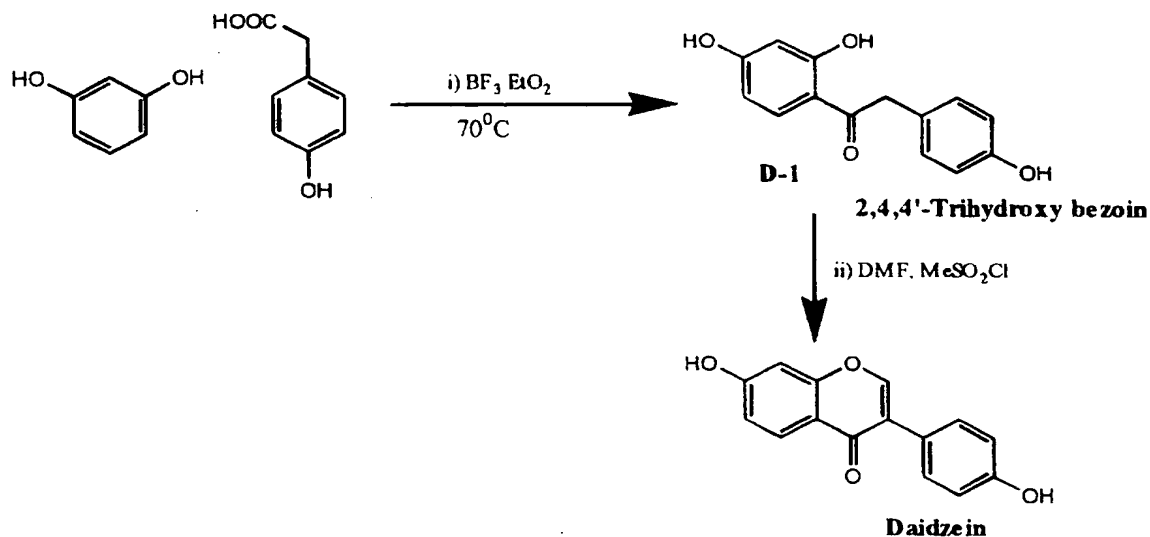
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3: Synthesis of Dihydrodaidzein (Compound 1)

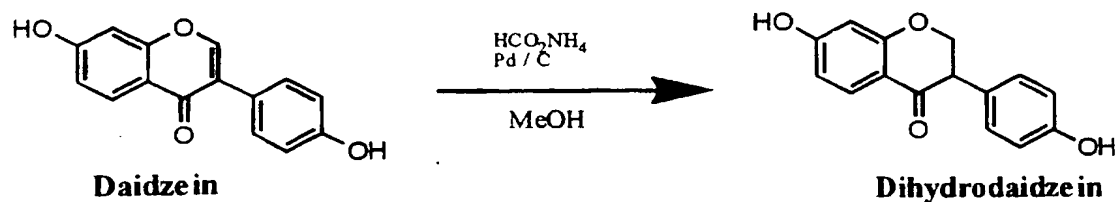
3.1 Synthesis of Daidzein



5

Resorcinol (29 mmol) and 4 hydroxyphenyl acetic acid (29 mmol) were dissolved into freshly distilled borontrifluoride etherate (20 mol eq) under nitrogen. The resulting mixture was stirred and heated at 70°C for overnight. The reaction was monitored by TLC(80% Et₂O / Hexane). The resulting mixture was cooled down at room temperature, then *N,N*-dimethylformamide (46.2 mL) was added dropwise. The mixture was again heated up at 50°C for 30 minutes, then methanesulphonyl chloride (7 mL in 10 mL DMF) was added dropwise and the resulting mixture was heated at 60 - 70° C until LC (80% Et₂O / Hexane) shown that the reaction was almost finished, about 10 hours. After cooling down at room temperature, the mixture was poured into 400 mL ice-cold water. The precipitate was filtered. The filtrate was collected and dried. The crude product was recrystallised in 94% ethanol (aq) and gave a quite pure daidzein (3g) in 44% yield.

3-2 Synthesis of dihydrodaidzein

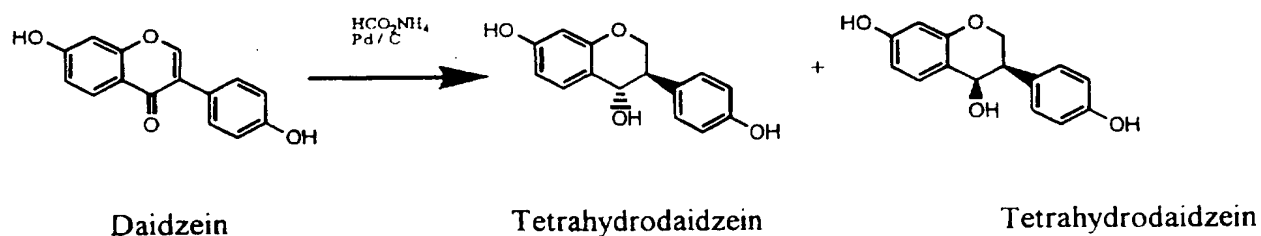


To a solution of daidzein (0.657g, 2.58 mmol) in methanol (60 mL) was added 10% Pd/C (0.657g) carefully, following by ammonium formate (0.652 g, 10.3 mmol). The mixture was heated up to 50-60°C and stirred for one hour. The reaction was monitored by TLC ($\text{CH}_2\text{Cl}_2/\text{EtOAc} = 7:2$ or 70% $\text{Et}_2\text{O}/\text{Hexane}$) and GC. After the reaction complete, the Pd/C was filtered and the filtrate was concentrated, which gave a crude product (0.558 g) of dihydrodaidzein as the major product and the *trans/cis* isomers of tetrahydrodaidzein as the minor products. The dihydrodaidzein was purified by standard procedures.

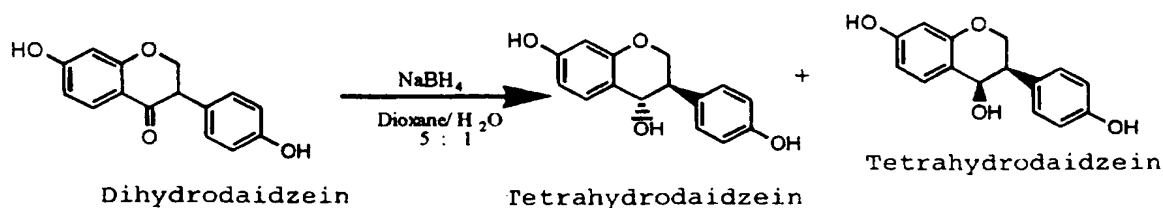
Other methods to provide dihydrodaidzein may be used such as that of Jain, A. C. and Mehta, A., J. Chem. Soc. Perkin Trans. I, 1986, 215.

4: Synthesis of the Tetrahydrodaidzein *trans* / *cis* isomers (Compound 8)

4-1 Synthesis of tetrahydrodaidzein *trans* / *cis*

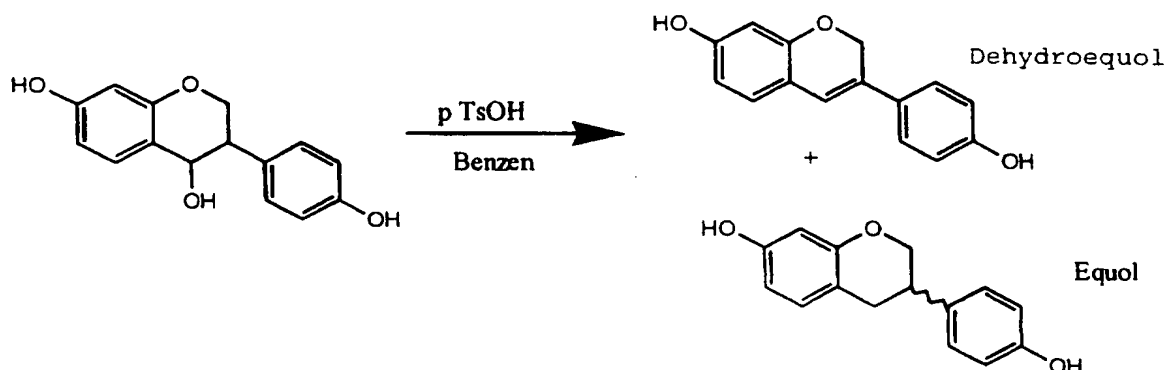


4-2 Synthesis of tetrahydrodaidzein *trans* / *cis*



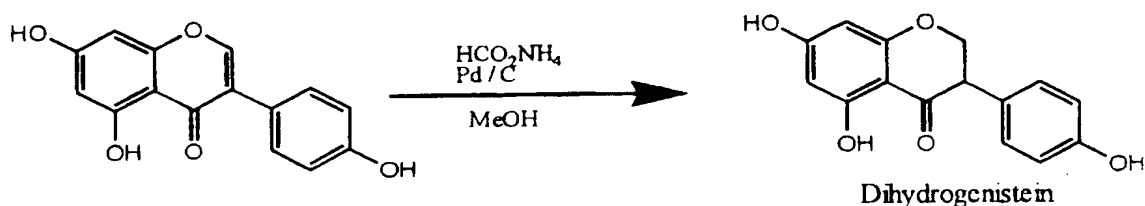
Dihydrodaidzein (0.001 g, 0.004 mmol) was dissolved in 200 L of dioxane and 40 L of water. Sodium borohydride (0.002 g, 0.053 mmol) was added and the resulting mixture was stirred at room temperature for two hours. Excess sodium borohydride was then destroyed with a drop of acetic acid and the mixture was evaporated to dryness by nitrogen. The residue was extracted with EtOAc and organic layer was washed with water and then evaporated to dryness. Gas chromatography showed that most of dihydrodaidzein was converted to the tetrahydrodaidzein as confirmed by GC-MS [M^+ 384. (G.E. Joannou, G.E.Kelly, A.Y.Reeder, M.Waring and C. Nelson. J.Steroid.Biochem. Molec. Biol. Vol.54, No 3/4, pp167-184,1995)]. Tetrahydrodaidzein was also synthesised by the reduction of dihydrodaidzein using sodium borohydride dioxane/ H_2O (5:1) (Ref: G.E. Joannou, G.E.Kelly, A.Y.Reeder, M.Waring and C. Nelson. J.Steroid.Biochem. Molec. Biol. Vol.54, No 3/4, pp167-184, 1995).

5: Synthesis of Dehydroequisol (Compound 10)



A mixture of tetrahydrodaidzein (0.02336g) was suspended in dry benzene (5 mL) and p-toluenesulfonic acid (0.0487 g) was added to the reaction. The resulting mixture was heated up 95°C for 35 min then the benzene was evaporated and the crude product was purified by the HPLC (MeOH/H₂O = 60 : 40) and gave dehydroequol and equol. The dehydroequol was confirmed by H NMR, GS-MS and high resolution MS.

6: Synthesis of Dihydrogenistein (Compounds 2 and 5)



10

Genistein (Sigma, 0.0023g, 0.0085 mmol) was dissolved in EtOH (2 mL) and 10% Pd/C (0.0023g) and ammonium formate (0.0027g, 0.043 mmol) were added to the solution with stirring. The resulting mixture was stirred overnight. GC showed that all the starting material was converted to dihydrogenistein as confirmed by GC, GC-MS and NMR data. The reduction product was purified by the HPLC.

15

EXAMPLE 4

The urine of volunteers is screened by way of gas chromatography-mass spectrometry (GC-MS) as described by Kelly *et al* in *Clinica Chimica Acta* (1993) 9-22, which is herein incorporated by reference). Those individuals whose urine contained greater than 0.5 µm and generally between 2.5 to 50 µm or more are selected for further study. Faecal samples were obtained from those individuals and microbial cultures made using standard faecal culture conditions. Microbial cultures which secrete the compounds of interest are detected by way of GC-MS. Organisms which secrete at least 50 µg of each of the compounds 1 through 19 were isolated. These organisms are used in microbial fermentation to produce compounds of the formulae 1 through 19. Where the organisms are selected from one of the classes

25

Lactobacilli, Clostridium perfringens, Bacteroids, Candida albicans and other yeasts, *Anaerobic cocci, Ruminococcus, Eubacterium, Peptostreptococcus, Clostridium, Bifidobacteria, Peptococcus, Streptococcus* and/or *Anaerobic streptococci, Gram-negative facultative bacteria, Fusobacterium* they may be used directly in food compositions such as dairy formulations so as to provide compounds of the formulae 1 to 19.

EXAMPLE 5

Therapeutic formulations were prepared by admixing compounds of the formulae 1 to 19 with a soy flour base (defatted soy flour available from Edible Enhanced Protein St Marys, Australia).

A range of pharmaceutical formulations is prepared comprising between 40 mg and 200 mg of active compound to a dosage formed.

For the purposes of this example, gelatin capsules and tablets containing 200 mg of each of the active compounds 1 to 19 are prepared in the soy flour base referred to above, or in a cholesterol free yoghurt base.

EXAMPLE 6

A. Treatment of vascular conditions - menopausal syndrome, hot flushes, hypertension, atherosclerosis and male impotency

Vascular reactivity studies using rat aortic rings is generally regarded as directly predictive of biological effects of candidate compounds in the treatment of the above conditions (Karapapanis, S. et al (1994) *Heptology*, 20, 6, 1516--1521). The inhibitory effect on restrictor responses in the aortic ring is measured in the presence of the vasoconstrictor noradrenaline according to the procedure of Karapapanis (supra). Dihydrodaidzein (Compound 1), dihydrogenestein (Compounds 2 and 5), tetrahydrodaidzein (Compound 8), ODMA (Compound 13) and equol (Compound 10) all exhibit potent inhibitory effects on responses to noradrenaline, that is, they inhibited vaso-constrictor responses.

Subsequent clinical studies are shown to demonstrate therapeutic benefits in the treatment of the above conditions using these compounds.

B. Hormone responsive cancer treatment - treatment of hormone related cancers including breast, ovarian, testicular, uterine, endometrial and prostatic cancer

The activity of compounds of the present invention in inhibiting the growth of hormone responsive cancer cells were tested using the well characterised human responsive cancer cell lines K562 and HL60. The anti-cancer screening assay measured inhibition of cell proliferation which results in terminal differential cell death. Cell death is due to either apoptosis or necrosis ODMA (Compound 13) and equol (Compound 10) or potent inhibitors of growth of cell lines K563 and HL60, this result being therefore directly predictive that these compounds will inhibit the growth of hormone related cancers such as those mentioned above. Tetrahydrodaidzein (Compound 8) showed strong inhibition of cell line HL60.

Subsequent clinical studies are shown to demonstrate therapeutic benefits in the treatment of the above conditions using these compounds.

C. Antioxidant studies - relevant to the treatment of cancer; conditions associated with oxidation of cholesterol such as atherosclerotic vascular disease; myocardial infarction, stroke, heart disease; arthritis and cataracts

Many studies have shown that compounds having antioxidant activity are useful therapeutics in the treatment of the above conditions (see for example McLaughlan *et al* (1995) *Biochem. Soc. Trans.* 23 (2) 2575; and van't Veer *et al* (1996) *Cancer Epidemiol Biomarkers Prev.* 5 (6) 441-7)

Compounds according to this invention have antioxidant activity.

Tetrahydrodaidzein (Compound 8) and dehydroequol (Compound 10) are highly effective antioxidants. The following tests in relation to these compounds are carried out:

1. LDL Antioxidation Test - This test measures the ability of a compound to directly scavenge free radicals or to chelate transition metals. The longer the lag time, the more active the compound as an antioxidant under these conditions compared to ascorbate as a positive control. These tests were carried out according to the procedure of Esterbauer et al *Free. Rad. Res. Coms.* (1989) 6, 67-75. Briefly, LDL (0.25 mg/ml) is incubated with 10 μ m active compound in the presence of 4 μ m Cu^{++} , and LDL was assayed for oxidation by HPLC analysis. Results are as follows:

	Sample	Lag Times - min	% Increase Over Control
10	Control	20	
	Ascorbate	50	150
	Tetrahydrodaidzein	>140	>600
	Dehydroequol	>140	>600

- 15 This significant finding shows that tetrahydrodaizein and dehydroequol are extremely potent antioxidants and therefore may be regarded as effective therapeutics in the treatment of cancer, myocardial infarction, stroke, arthritis, sunlight induced skin damage cataracts, and other conditions resulting from oxidative damage.

- 20 2. Redox Test - This test measures the ability of a compound to prevent LDL lipid oxidation in the presence of vitamin E. The test is a physiological test, vitamin E (α -tocopherol) is present with LDL in the blood stream, and LDL oxidation is believed to be one of the major factors of the development of atherosclerosis. The lower values, the higher the redox activity. A high redox activity suggests that the compound is
- 25 capable of interacting with the α -tocopherol in the LDL, perhaps by reducing the α -tocopheroxyl radical. The test indirectly assesses the ability of a compound to synergise with α -tocopherol in human LDL undergoing mild and chemically controlled oxidation. Oxidation is measured by the accumulation of cholesterylester hydroperoxides at a time point corresponding to 20% consumption of endogenous α -
- 30 tocopherol. Butylated hydroxytoluene (BHT 10 μ m) is used as a positive control. The redox index is measured by the relative extent of oxidation of LDL in the presence of

the sample divided by the relative extent of oxidation in the absence of the test compounds. Active compounds give rise to low Redox Index. Tests were carried out according to Bowry, V.W. *et al* (1995) *J. Bio. Chem.* 270 (11) 5756-5763. Such tests show that compounds 1 to 19 synergistically interact with vitamin E to prevent oxidation of lipids, proteins, and other biological species.

By way of example dehydroequol (Compound 10) tested in this assay is shown to be a particularly superior antioxidant compared to a positive control antioxidant (BHT), the Redox Index for dehydroequol being 4.5 ± 1.2 , and that of BHT being 6.3.

The above test indicates that compounds 1 to 19, and particularly dehydroequol interacts synergistically with vitamin E to prevent oxidation. This is an important finding as vitamin E has previously been regarded as having opposing activities facilitation oxidation and decreasing oxidation of lipids and protein. Compositions containing one or more Compounds 1 to 19 and vitamin E may be used in the therapeutic treatment of cancer, myocardial infarction, stroke, arthritis, sunlight induced skin damage cataracts and other conditions responsive to treatment with antioxidants.

3. Synergism with α -tocopherol (TRAA) - This test directly assesses the ability of the test sample to attenuate α -tocoperoxyl radicals in cetyltrimethyl ammonium chloride (HTAC) or SDS micelles. Ascorbate is used as a positive control. Results are expressed as the relative rate constant of decay of α -tocoperoxyl radicals in the presence of the test sample divided by the relative rate constant of decay of α -tocoperoxyl radicals in the absence of the test sample. TRAA approaching unity is considered to have poor synergistic activity, whereas active compounds show large values because they eliminate the α -tocoperoxyl radicals immediately upon mixing.

The experiments were carried out according to Witting *et al* (1996) *J. Lipid Res.*, 37, 853-867. These studies show that Compounds 1 to 19, particularly dehydroequol (Compound 10), dihydrodaidzein and dihydrogenistein, interact synergistically with α -tocopherol.

4. LDL Receptor Studies - Treatment of atherosclerosis, myocardial infarction, stroke and hypertension. This will establish that compounds which up-regulate the LDL receptor, lead to decreased circulating LDL, and therefore reduces the prospect of atherosclerosis, myocardial infarction, stroke and hypertension. Using an assay according to Stephan Z.F. and Yurachek, E. C. (1993) *J. Lipid. Res.* 34, 325-330, it is shown that the compounds of the formulae 1 to 19 are effective in increasing LDL uptake into liver cells, this being directly predictive of decrease of circulating LDL in the human blood stream. ODMA and equol are shown to be particularly active in this respect.

EXAMPLE 7

Treatment of acne

An 18 year old girl with acne since puberty, with no response to the contraceptive pill or any topical cream, and who declined the use of Roacutane on safety grounds, was administered a soy isoflavone extract containing genistein, daidzein, formononetin and Biochanin A which were converted into their metabolites namely compounds 1, 2, 5, 8, 10, 11, 13 and 14 as evidenced by urine analysis. 40 mg administered twice daily resulted in marked improvement of acne condition, colour, and general appearance within two weeks.

A 40 year old man with acne since puberty, with no response to any topical cream and who declined the use of Roacutane on safety grounds, as administered a soy isoflavone extract as described above. These isoflavones were converted to their metabolites namely compounds 1, 2, 5, 8, 10, 11, 13 and 14 as evidenced by urine analysis. Unexpectedly, he reported a dramatic improvement in his acne within two weeks, a change which hadn't been observed in over 20 years.

Subsequent clinical studies have shown to demonstrate therapeutic benefits in the treatment of acne utilising the above compounds.

Subsequent clinical studies are shown to demonstrate therapeutic benefits in the treatment of the above conditions.

EXAMPLE 8

5 A 67 year old man suffering from prostate cancer received a daily dosage of 16 mg of isoflavone extracts from clover which contained genistein, daidzein, formononetin and biochanin A. After subsequent surgery for his prostate cancer condition the pathology report on the extracted prostate tissue showed an increased incidence of apoptosis (Stephens, F. O. (1997) *J. Aus. Med. Assoc.* 167, 3, 138-140). Analysis of this patient's urine showed the presence of aforementioned metabolites, this indicating that these compounds responsible for
10 the amelioration of his condition in that the degenerative changes in the prostatectomy section, especially the apoptosis were indicative of androgen deprivation and typical of a response to estrogen therapy.

EXAMPLE 9

15 A patient group was studied comprising women who had a past history of breast cancer (who had been treated either by surgery or radiation, or both) and women who had a strong familial connection to breast cancer, that is, where their mothers or siblings had suffered from breast cancer. This study investigated whether compounds 1 through 19 administered transdermally each day through a skin patch could be used to prevent breast cancer or metastatic cancers following cancer therapy.

20

Patches were prepared that contained a lipophylic carrier cream which is readily absorbed through the skin. The cream comprised a glycerol cold cream which contained glycerin and peanut oil. A selected active compound from any one of compounds of the formulae 1 to 19 is mixed with the lipophylic cream such that each patch comprises 10 mg to 100 mg of active
25 compound. The patch is applied to the skin each day and rapid absorption occurs. After two hours the patch is removed. Alternatively, the patch may be left on for a greater part of each day.

Over a one year study period it is found that this high risk group does not show any evidence
30 of breast cancer or other metastatic cancer.

The effectiveness of this treatment is shown in another study of a similar group of high risk patients. Compounds 11, 13 and 14 are transdermally administered to patients in the same manner and amount as above. The same beneficial results are observed over a six month trial period.

5

EXAMPLE 10

A study of a group of patients suffering from benign prostatic hypertrophy (BPH) and prostatic cancer of various grades is carried out to determine the effect of administration of compounds of the formulae 1 to 19. The administration protocol was the same as for Example 3 involving
10 daily administration of a gelatin capsule containing 200 mg of active compound. A significant decrease in the rate of production of relevant cancer markers (PSA, prostate specific antigen) is observed. Tumours are again shown to have regressed, or show no further growth. In another study a 45 year old male with BPH presented with urinary obstruction and frequency of urination. Upon taking 40 mg per day of a clover isoflavone containing extract became
15 symptom free. Urine analysis showed the presence of the urinary metabolites described above.

A patient suffering from advanced bowel cancer is treated daily for three weeks with an intravenous infusion of 2 g of the compound of the formula 14 dissolved in sterile saline. The patient's pain and discomfort was significantly reduced, and reduction in cancer markers is
20 observed. Progression of the tumour is also arrested over the treatment period.

A second patient suffering from the same condition is treated in the same manner as the above patient with the exception that the 2 g dose of the active compound is administered by way of bolus injection. The results obtained were the same as those discussed in the above paragraph.

25

In a further series of experiments a group of patients suffering from terminal bowel cancer were treated by bolus daily injections (intravenous or intramuscular) of 2 g of a compound selected from one of the formulae 1 to 19. Over the test period there is shown to be a marked reduction in pain and discomfort. Tumour markers (carcino-embryonic antigen (CEA)) are reduced as
30 evidenced by blood analysis and tumour spread decreased.

EXAMPLE 11

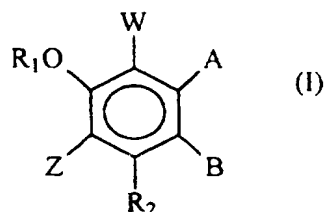
A study of patients suffering from male pattern baldness was carried out. Each of the subjects received a daily application to the scalp of an inert pharmaceutical gel containing 50 mg of active material. Over the one month study period there is observed a light down or stubble appearing on the treated area. This study indicates that the compounds are effective in the treatment of hair loss and with long term application should provide hair regeneration.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising" or the term "includes" or variations thereof, will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers. In this regard, in construing the claim scope, an embodiment where one or more features is added to any of claim is to be regarded as within the scope of the invention given that the essential features of the invention as claimed are included in such an embodiment.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications which fall within its spirit and scope. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

The claims defining the invention are as follows:

1. A method for the treatment of prophylaxis, amelioration, defence against, and/or prevention of menopausal syndrome including hot flushes, anxiety and depression; mood swings; night sweats; headaches; urinary incontinence; osteoporosis; premenstrual syndrome; fluid retention; dysmenorrhoea; Reynaud's Syndrome; Reynaud's Phenomenon; Buerger's Disease; coronary artery spasm; migraine headaches; osteoporosis, hypertension, benign prostatic hypertrophy; breast cancer; uterine cancer; ovarian cancer; testicular cancer; large bowel cancer; endometrial cancer; prostatic cancer; cyclical mastalgia; uterine cancer; atherosclerosis; Alzheimers disease; inflammatory diseases including inflammatory bowel disease, ulcerative colitis, Crohns disease; rheumatic diseases including rheumatoid arthritis; acne; baldness including male pattern baldness (alopecia hereditaria); psoriasis and diseases associated with oxidant stress including cancer, myocardial infarction stroke, arthritis sunlight induced skin damage or cataracts; which comprises administering to a subject a therapeutically effective amount of one or more compounds of the formula I:



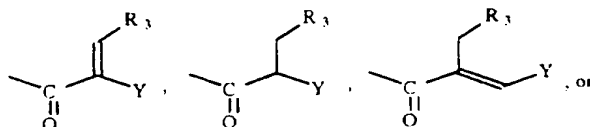
in which

Z is H,

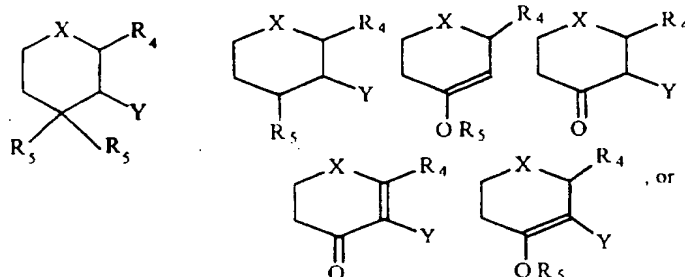
R₁ is H, or R_ACO where R_A is C₁₋₁₀ alkyl or an amino acid,

R₂ is H, OH, or OR_B where R_B is an amino acid or COR_A where R_A is as previously defined,

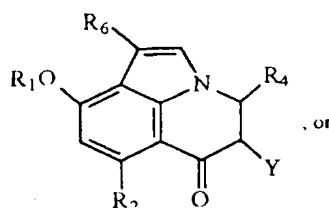
W is H, A is H or OH, and B is selected from



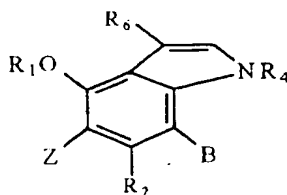
W is H, and A and B taken together form a six membered ring selected from



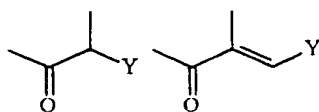
W, A and B taken with the groups with which they are associated comprise



W and A taken together with the groups with which they are associated comprise



and B is



wherein

R_3 is H, COR_A where R_A is as previously defined, CO_2R_C where R_C is C_{1-10} alkyl, or COR_B where R_B is as previously defined,

R_4 is H, COR_D where R_D is H, OH, C_{1-10} alkyl or an amino acid, CO_2R_C where R_C is as previously defined, COR_E where R_E is H, C_{1-10} alkyl or an amino acid, COOH, COR_C where R_C is as previously defined, or $CONHR_E$ where R_E is as previously defined,

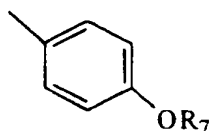
R_5 is H, CO_2R_C where R_C is as previously defined, or

$\text{COR}_\text{C}\text{OR}_\text{E}$ where R_C and R_E are as previously defined, and where the two R_S groups are attached to the same group they are the same or different,

R_G is H or hydroxy C_{1-10} alkyl,

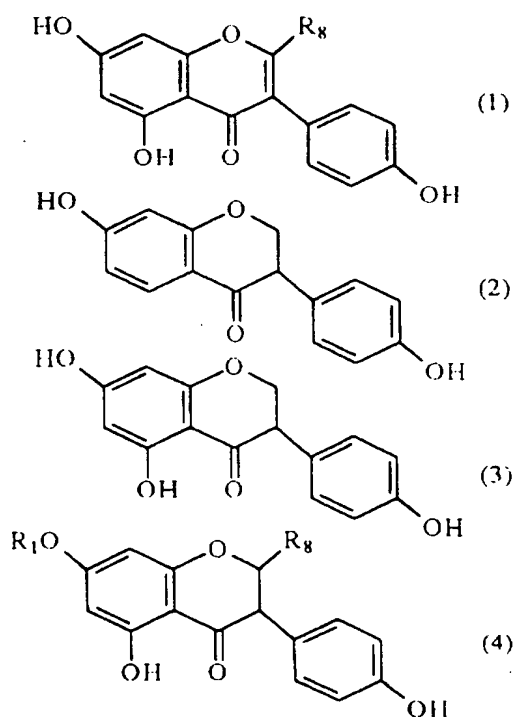
X is preferably O, but may be N or S, and

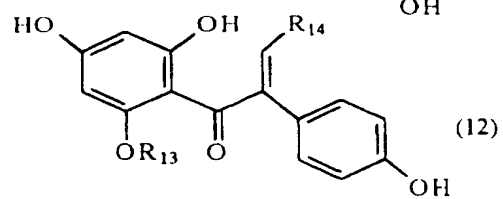
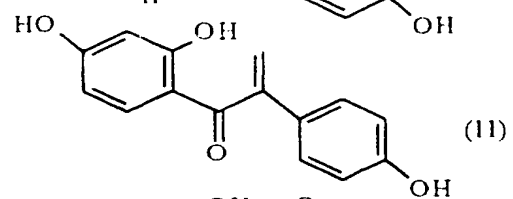
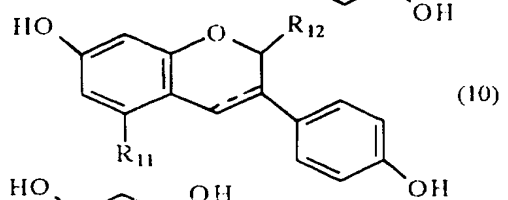
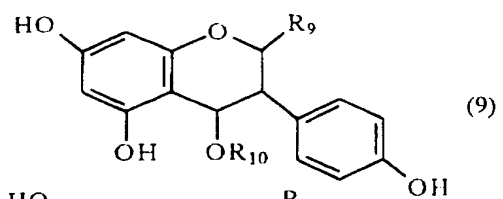
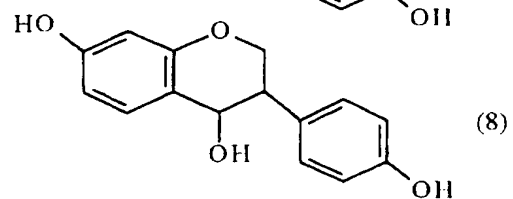
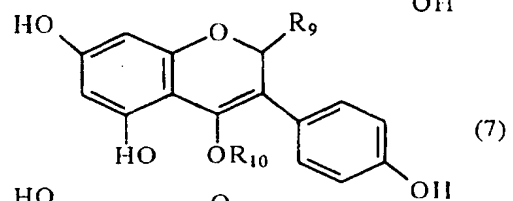
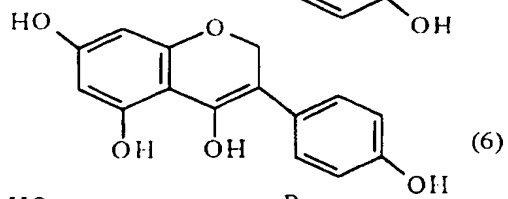
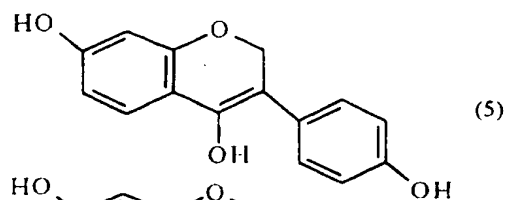
Y is

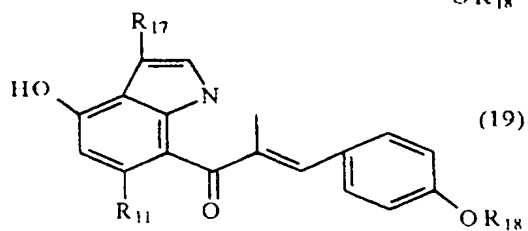
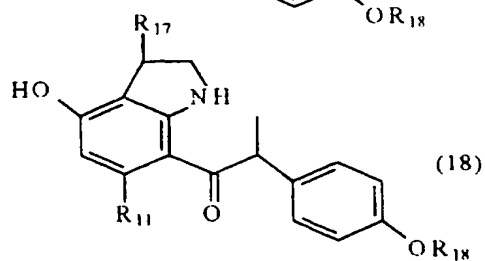
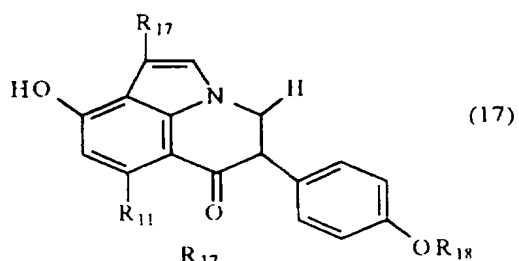
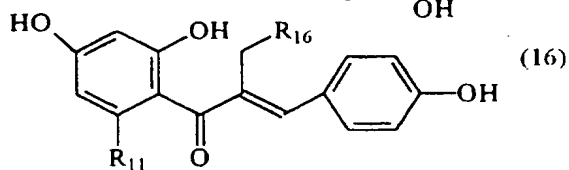
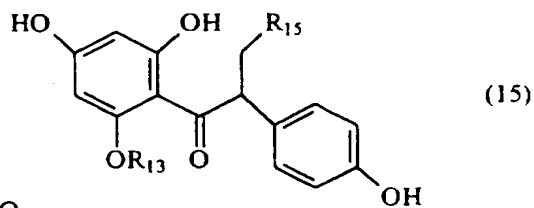
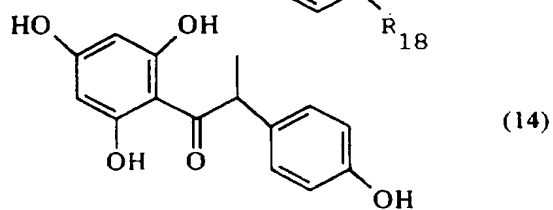
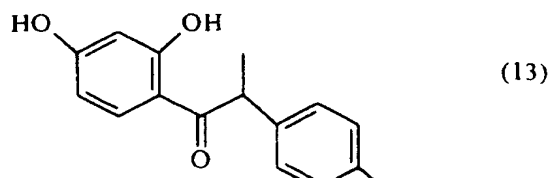


where R_7 is H, or C_{1-10} alkyl.

2. A method according to claim 1 wherein said compounds of the formula I are selected from:







wherein

R_g is COR where R_D is as previously defined,

R_e , CO_2R_C or COR_E where R_C and R_E are as previously defined,

R_{10} is COR_C or COR_COR_E where R_C and R_E are as previously defined,

R_{11} is H or OH,

R_{12} is H, COOH, CO_2R_C where R_C is as previously defined, or $CONHR_E$ where R_E is as previously defined,

R_{13} is OH, OR_B where R_B is as previously defined, or COR_A where R_A is as previously defined,

R_{14} is H, or COR_A where R_A is as previously defined,

R_{15} is COR_A where R_A is as previously defined,

R_{16} is H, COR_B or CO_2R_C where R_B and R_C are as previously defined,

R_{17} is H or hydroxy C_{1-10} alkyl,

R_{18} is H or C_{1-10} alkyl,

and "---" represents either a single bond or a double bond.

3. A method according to claim 2 which is a method for the treatment of menopausal syndrome, hypertension, atherosclerosis, male impotence, premenstrual syndrome, or cyclical mastalgia wherein one or more compounds of the formulae 1, 3, 8, 10, 11, 13 or 14 are administered to a subject in need of such treatment in association with a pharmaceutically acceptable carrier or excipient.
4. A method according to claim 2 which is a method for the treatment of breast, ovarian, testicular, uterine, large bowel, leukemia, endometrial, or prostatic cancer wherein one or more compounds of the formulae 1, 8, 10, 11, 13 or 14 are administered to a subject in need of such treatment in association with a pharmaceutically acceptable carrier or excipient.
5. A method according to claim 2 which is a method for the treatment of cancer, myocardial infarction, stroke, arthritis or cataracts which comprises administering to a subject in need of such treatment one or more compounds of the formulae 1, 3, 8, 10, 13 and 14 in association with a pharmaceutically acceptable carrier and/or excipient.

6. Use of the compounds of the formula I for the manufacture of a medicament for the treatment, amelioration, defence against prophylaxis and/or prevention of one or more of the therapeutic indications set forth in claim 1.
7. Use according to claim 6 wherein said compounds of the formula I are selected from compounds 1 to 19 as defined in claim 2.
8. Use of a compound of the formula I in the treatment, amelioration defence against, prophylaxis and/or prevention of one or more of the therapeutic indications set forth in claim 1.
9. Use according to claim 8 wherein said compounds of the formula I are selected from compounds 1 to 19 as defined in claim 2.
10. An agent for the treatment, prophylaxis, amelioration, defense against and/or treatment of one or more of the therapeutic indications set forth in claim 1.
11. An agent according to claim 10 wherein said compounds of the formula I are selected from compounds 1 to 19 as defined in claim 2.
12. A composition which comprises one or more compounds of the formula I in association with one or more pharmaceutically carriers and/or excipients.
13. An antioxidant composition which comprises one or more compounds of the formula I in association with one or more physiologically and/or cosmetically acceptable carriers and/or excipients.
14. An antioxidant composition which comprises one or more compounds of the formula I, and vitamin E, optionally in association with a pharmaceutically acceptable carrier and or excipient.

SUBSTITUTE SHEET (RULE 26)

15. An antioxidant composition according to claim 14 wherein said one or more compounds of the formula I comprise one or more compounds of the formulae 1 to 19 as defined in claim 2.
16. A composition according to claim 13 which comprises a skin cream or gel which contains one or more of compounds 1 to 19 as defined in claim 2.
17. A composition according to claim 13 which comprises a solid dosage unit composition comprising one or more compounds of compounds 1 to 19 as defined in claim 2.
18. A composition according to claim 12 wherein said compounds of the formula I are selected from compounds 1 to 19 as defined in claim 2.
19. A food stuff or drink which contains one or more compounds of the formula I.
20. A food stuff or drink according to claim 17 wherein said compounds of the formula I are selected from compounds 1 to 19 as defined in claim 2.
21. A microbial culture or a food stuff containing one or more microbial strains, which microorganisms produce one or more compounds of the formula I.
22. A microbial culture according to claim 21 wherein said compounds of the formula I are selected from compounds 1 to 19 as defined in claim 2.
23. A microorganism which produces one or more compounds of the formula I, or a mixture of microorganisms which produce one or more compounds of the formula I.
24. A microorganism according to claim 23 wherein said compounds of the formula I are selected from compounds 1 to 19 as defined in claim 2.

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 97/00563

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl⁶: A61K 31/35, 31/12, 31/40, 31/475

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: A61K 31/35, 31/12, 31/40, 31/475, C12P 17/06, C12N 1/20

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DERWENT: ISOFLAV: PHYTOEST., ESTROG., OESTROG., PHYTOOESTROGEN
CHEMICAL ABSTRACTS: sub structure search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93/23069 A (KELLY, Graham Edmund) 25 November 1993 entire document	1-24
X	WO 96/10341 A (SCHOUTEN INDUSTRIES B.V.) 11 April 1996 entire document	1-24
X	WO 94/23716 A (THE UNIVERSITY SCHOOL OF MEDICINE) 27 October 1994 entire document	1-24

☒ Further documents are listed in the continuation of Box C

☒ See patent family annex

<p>* Special categories of cited documents:</p>	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier document but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
26 September 1997

Date of mailing of the international search report

03 OCT 1997

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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 97/00563

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 135172 A2 (TAKEDA CHEMICAL INDUSTRIES, LTD) 27 March 1985 entire document	1-24
X	US 5424331 A (SHLYANKEVICH) 13 June 1995 entire document	1-24
X	US 5523087 A (SHLYANKEVICH) 4 June 1996 entire document	1-24
X	DE 4432947 A1 (NEW STANDARD GMBH) 21 March 1996 entire document	1-24
X	Derwent Abstract Accession No. 95-272884, Class B03, JP 0517056 A (KIKKOMAN CORP) 9 July 1993 entire document	1-24
X	Derwent Abstract Accession No. 93-252721, Class B02, JP 07173148 A (KIKKOMAN CORP) 11 July 1995 entire document	1-24
X	Derwent Abstract Accession No. 86-329949, Class B02, JP 61247396 A (YAMANOUCHI PHARM KK) 4 November 1986 entire document	1-24
X	Patent Abstract of Japan, C-452, page 118, JP 62-106016 A (YAMANOUCHI PHARMECEUT CO LTD) 16 May 1987 entire document	1-24
X	Patent Abstract of Japan, C-452, page 118, JP 62-106017 A (YAMANOUCHI PHARMACEUT CO LTD) 16 May 1987 entire document	1-24
X	Patent Abstract of Japan, C-722, page 98 JP 2-67218 A (NAGAKURA SEIYAKU KK) 7 March 1990 entire document	1-24
X	Kelly G. <i>et al.</i> , 'Metabolites of dietary (soya), isoflavones in human urine', <i>Clinica Chimica Acta</i> 223 (1993) 9-22 entire document	1-24
X	Joannou G. <i>et al.</i> , 'A urinary profile study of dietary phytoestrogens. The identification and mode of metabolism of new isoflavanoids' <i>J. Steroid Biochem. Molec. Biol.</i> 54 (3/4) (1995) 167-184 entire document	1-24
X	US 5352384 A (SHEN) 4 October 1994 entire document	1-24
X	WO 97/06273 (FERMALOGIC INC) 20 February 1997 entire document	1-24

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.
PCT/AU 97/00563

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
(To put a line under the citations tab to the first point on the next row and press F8)							
WO	9323069	AU	40525/93	EP	656786	NO	944435
		NZ	252051				
WO	9610341	AU	36890/95				
WO	9423716	AU	66359/94	CA	2160371	EP	693927
		US	5498631				
EP	135172	DE	3430799	IT	1179067	JP	60048924
WO	9706273	AU	67161/96	US	5554519		
END OF ANNEX							

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